

28. The method according to claim 27, wherein the linker has a length of 4 to 7 carbon atoms.

a2
29. The method according to claim 28, wherein the linker has a length of 4 carbon atoms.

REMARKS

This application is a national stage of PCT/US00/09512. In the International Preliminary Examination Report issued by the IPEA/US the Examiner indicated that claims 3-7 and 9-14 met the requirements of PCT Article 33(2)-(4). This amendment is filed to present only those claims.

Claims 1 and 2 have been cancelled, and claim 3 has been amended to independent form. Claim 5 has been cancelled as a duplicate of claim 9, and claim 6 has been amended to avoid dependency on a cancelled claim. Claim 8 has been cancelled and claim 9 has been amended to avoid dependency on a cancelled claim.

Claims 12 and 13 have been amended to include the recitation of the chemical composition from claim 3 and to eliminate multiple dependency. Claims 15-29 have been added to add the limitations of the dependent composition claims which had previously been included as a result of multiple dependency.

In addition, Applicants have made some minor amendments to the specification. These amendments add a reference to the parent PCT application, and correct several grammatical and clerical errors. No new matter is added by these amendments.

With respect to the replacement figure on Page 3, the replacement adds a double bond which was inadvertently omitted from one of the geldanamycin moieties, but which is included in the other. Since the specification clearly identifies the two parts of this molecule as being the same, and since the structure of geldanamycin was known, this is merely a clerical correction and is not new matter.

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Applicants also enclose proposed replacement sheets for Figures 1 and 2, on which the proposed changes are highlighted. In Fig. 1, the changes include the addition of the missing double bond as noted above, changing the view so that the amide groups are more clearly depicted, and adding a subscript to the reagent $\text{H}_2\text{N}(\text{CH}_2)\text{NH}_2$ so that it correctly reads $\text{H}_2\text{N}(\text{CH}_2)_7\text{NH}_2$. The person skilled in the art would understand that a subscript had been omitted, and that the correct value is 7 since the product formed in the figure has a seven carbon linker. In Fig. 2, the changes alter the view so that the amide groups are more clearly depicted, and expand the carboxyl groups. It also indicates omitted orientation on one hydroxyl group, consistent with the orientation in the parent molecule. No new matter is added by these corrections.

In view of the indication that all of the claims now pending meet the requirements of PCT Article 33(2)-(4), Applicants submit that this application should be in form for allowance. Expedited handling in accordance with the provisions 37 CFR § 1.496(b) is respectfully requested.

Respectfully submitted,

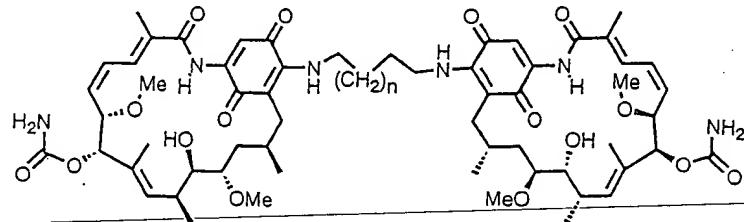
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Marked Up Copy Showing Amendments

On Page 2 of the specification:

HER-family transmembrane receptor tyrosine kinases play an important role in transducing extracellular growth signals and when activated can be oncogenic. Tzahar, et al., *Biochim Biophys Acta* 1377, M25-37 (1998); Ross, et al., *Stem Cells* 16, 413-428 (1998). Overexpression of HER1 and HER2 occurs in a variety of human malignancies. Amplification of the HER2 gene is a common event in human breast and other carcinomas and, in breast cancer, is associated with a poor prognosis[2]. HER1 and HER2 are attractive targets for therapeutic development. Antibodies against each of these receptors have been shown to have antitumor effects in animal models. Fan, et al., *Curr Opin Oncol* 10, 67-73 (1998). Recently, an anti-HER2 antibody was shown to be effective in the treatment of breast cancers in which the HER2 protein is overexpressed. Ross et al., *supra*; Pegram, et al., *J Clin Oncol* 16, 2659-2671 (1998). However, therapeutic effects were seen in only a minority of patients and were usually short-lived. It is not known whether this is due to

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Dimers: n = 1- 9

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cause them to undergo homodimerization or heterodimerization with other members of the family. This activates the tyrosine kinase activity of the constituents of the dimer, causes their autophosphorylation and initiates transduction of the mitogenic signal. Although a direct interaction of hsp90 and HER-kinases has not been convincingly demonstrated, the fact that sensitivity of HER2 and other kinases to geldanamycin requires the catalytic domain of the kinase suggests that hsp90 is likely to interact with the catalytic domain of HER-kinases. As HER-kinase heterodimers are quite sensitive to GM, we speculated that each element of the heterodimer interacts with hsp90. Accordingly, it is believed that the dimers of the invention interact with both subunits of the HER-kinase heterodimers and thus more effectively and specifically target the active form of the HER-kinase. The mechanism of action [is] appears to

be based [to at least] on degradation of the HER-kinases, but may include or in some cases be derived entirely from an inhibition of activity of the HER-kinases.

Fig. 2 shows various compounds which have been synthesized and tested for activity and selectivity as promoters of tyrosine kinase degradation. The compounds tested included geldanamycin, geldanamycin homodimers with linkers of varying lengths, species with quinone or ring[ed]-opened geldanamycin linked to geldanamycin and geldanamycin coupled to a linker with no substituent at the other end. The linker in each case is bonded to [the 17-carbon] carbon-17 of the geldanamycin moiety or moieties. The crystal structure of GM bound to hsp90 shows that [the 17-carbon] carbon-17 is the only one not buried in the binding pocket. Stebbins, et al., *Cell* 89, 239-250 (1997).

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GMD-4c was also a potent inhibitor of the growth of breast cancer cells containing HER-kinases (Table 1) with an IC₅₀ of 100 nM against MCF-7 compared to IC₅₀ 25 nM for GM and 650 nM for the one-ring opened dimer GMD-a. SKBR3 in which HER2 is highly overexpressed was also found to be very sensitive to GMD-4c. Most epithelial cancer cell lines express one or more members of the HER-kinase family. In order to assess whether the effects of GMD-4c on cells were specific, we utilized the 32D hematopoietic cell line. None of the members of the HER-kinase family are expressed in this murine IL3-dependent myeloid progenitor cell line. Wang, et al., *Proc Natl Acad Sci (USA)* 95, 6809-6814 (1998). GM is a potent inhibitor of 32D; GMD-4c does not [appreciable] appreciably affect its growth.

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Based on these experimental results, we conclude that GMD-4c induces the selective-degradation and/or inhibition of HER-family kinases and [the] specifically inhibits the growth of HER-kinase containing tumor cell lines. This work[s] supports the idea that selective ansamycins with a different, more restricted spectrum of targets than the parent molecules can be synthesized. In this case, the mechanism of selectivity is not yet known, but depends on the presence of both GM moieties and is a function of the length of the linker. GMD-4c may selectively interact with HER-kinase heterodimers, but it is also possible that it preferentially interacts with different hsp90 family members than GM.

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100% dimethylsulfoxide (DMSO) and stored at -20°C prior to use in preparation of the geldanamycin derivatives of Fig. 2. The GM analogs were prepared according to the procedure depicted in Fig. 1, which is modified from the method of Schnur et al., *J. Med. Chem.* 38, 3813-3820 (1995). Briefly, the geldanamycin dimers (GMDs) were prepared by treatment of GM with 0.5 eq. of the appropriate diamine in DMSO. The ansa ring-opened [ansaring-opened] GMDs (GMD-a and GMD-aa) were prepared by methanolysis (NaOMe/MeOH) of the GMD-4c. GM-

quinone was synthesized by first treating GM with excess 1,4-diamobutane, then addition of 2-methoxy-1-hydroxymethylquinone.

In the Figures:

Please replace figures 1 and 2 with the enclosed replacement sheets.

In the claims:

3. (amended) A [The] chemical compound [according to claim 2] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.

6. (amended) The chemical compound of claim [5] 4, wherein the linker has a length of 4 to 7 carbon atoms.

9. (amended) The chemical compound of claim [8] 3, wherein the first and second hsp-binding moieties are geldanamycin.

12. (amended) A method for destruction of cells expressing a HER-family tyrosine kinase, comprising administering to the cells a chemical compound [according to any of claims 1-11] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.

13. (amended) A method for treating cancer in a patient[s] suffering from cancer, comprising administering to the patient a therapeutic composition comprising a chemical compound [according to any of claims 1-11] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.